DCIEM 98-P-51

Low doses of melatonin and diurnal effects on thermoregulation and tolerance to uncompensable heat stress

TOM M. McLELLAN, GREG A. GANNON, JIRI ZAMECNIK, VALERIE GIL, AND GREG M. BROWN
Human Protection and Performance Section, Defence and Civil Institute of Environmental Medicine, Toronto, Ontario M3M 3B9, Canada

McLellan, Tom M., Greg A. Gannon, Jiri Zamecnik, Valerie Gil, and Greg M. Brown. Low doses of melatonin and diurnal effects on thermoregulation and tolerance to uncompensable heat stress. *J. Appl. Physiol.* 87(1): 308–316, 1999.—This study examined whether the reported hypothermic effect of melatonin ingestion increased tolerance to exercise at 40°C, for trials conducted either in the morning or afternoon, while subjects were wearing protective clothing. Nine men performed four randomly ordered trials; two each in the morning (0930) and afternoon (1330) after the double-blind ingestion of either two placebo capsules or two 1-mg capsules of melatonin. Despite significant elevations in plasma melatonin to over 1,000 ng/ml 1 h after the ingestion of the first 1-mg dose, rectal temperature (T_{re}) was unchanged before or during the heat-stress exposure. Also, all other indexes of temperature regulation and the heart rate response during the uncompensable heat stress were unaffected by the ingestion of melatonin. Initial T_{re} was increased during the afternoon (37.1 ± 0.2°C), compared with the morning (36.8 ± 0.2°C) exposures, and these differences remained throughout the uncompensable heat stress, such that final T_{re} was also increased for the afternoon (39.2 ± 0.2°C) vs. the morning (39.0 ± 0.3°C) trials. Tolerance times and heat storage were not different among the exposures at ~110 min and 16 kJ/kg, respectively. It was concluded that this low dose of melatonin had no impact on tolerance to uncompensable heat stress and that trials conducted in the early afternoon were associated with an increased T_{re} tolerated at exhaustion that offset the circadian influence on resting T_{re} and thus maintained tolerance times similar to those of trials conducted in the morning.

rectal temperature; heat storage; protective clothing; circadian rhythm

IN PRESENT INDUSTRIAL AND MILITARY SETTINGS, personnel may be required to wear protective clothing to maintain work schedules in a hazardous environment. Typically, the protective clothing has a reduced water vapor permeability. Thus the clothing limits the evaporation of sweat, thereby increasing the rate of heat storage for a given rate of heat production. Uncompensable heat stress develops when the evaporative heat loss, required (E_{req}) to maintain a thermal steady state, exceeds the maximum evaporative potential (E_{max}) of the environment. It is not uncommon, therefore, for conditions that would normally be defined as compensable heat stress, to become uncompensable when protective clothing is worn (23). The heat strain associated with wearing the military's current-issue nuclear, biological, and chemical (NBC) protective clothing is well documented for many countries at different ambient temperatures, vapor pressures, and metabolic rates (1, 9, 18, 21, 29, 31, 34).

Tolerance time while subjects are wearing NBC protective clothing is determined from the initial core temperature, the rate of heat storage, and the final core temperature tolerated at exhaustion. Unless cooling is provided, there is very little that can be done when the current NBC protective clothing is worn to alter the rate of heat storage or the core temperature tolerated at exhaustion, as demonstrated after glycerol or water hyperhydration (27), water rehydration during the heat stress (10), aerobic training (2, 12), or heat acclimation (3, 11, 30). However, factors such as aerobic fitness (11) and heat acclimation (3, 30) lower the initial core temperature 0.2–0.3°C and, therefore, increase tolerance time, whereas factors such as mild hypohydration (11, 12) or the postovulatory phase of the menstrual cycle (22, 42) raise the starting core temperature and decrease tolerance time. The extent to which tolerance time is altered depends on the severity of the heat stress index, defined as the ratio of E_{req} to E_{max}, with greater changes being observed at lighter metabolic rates that are defined by a lower heat stress index (2, 3, 10).

The normal circadian rhythm leads to oscillations in resting core temperature that can vary by 0.5°C from early morning to midafternoon (25). If the core temperature tolerated at exhaustion does not similarly show a circadian effect, and there is no evidence to suggest that it does, then the higher resting core temperatures in the afternoon should reduce tolerance time while subjects are wearing protective clothing. This potential circadian influence has not been documented for the uncompensable heat stress conditions defined by wearing the NBC protective clothing and exercising in hot environments.

Melatonin is secreted by the pineal gland under the control of the suprachiasmatic nuclei with a circadian rhythm that follows our normal light and dark hours. Melatonin blood levels begin to increase at ~2000, reaching peak levels early in the morning at ~0300 (5). Thereafter, levels begin to decrease, reaching baseline daylight values at ~0800 (5, 6). Although a cause-and-effect relationship has not yet been proven, the circadian rhythm for core temperature is inversely related to these changes in melatonin (5). For example, as melatonin levels begin to increase, core temperature begins to fall, reaching its lowest value when melatonin levels peak. In addition, oral ingestion of melatonin can suppress the normal rise in core temperature that occurs during the morning and early afternoon daylight hours (6). Furthermore, oral ingestion of melatonin can invoke the normal circadian drop in core temperature in the evening if someone is exposed to bright light (i.e.,

8750-7587/99 $5.00 Copyright © 1999 the American Physiological Society

102-00766/

shift workers) (41). Within 60–90 min after a 1-mg oral dose of melatonin, Dawson et al. (13) observed a fall in core temperature approximating 0.25°C for up to 3 h. The change in core temperature appears to be related to a threshold level of melatonin being present in the blood rather than a dose-response relationship (7). However, doses <1 mg appear to provide only a small effect on core temperature (13). In theory, therefore, ingestion of melatonin before heat exposure in the afternoon may suppress the negative impact of higher afternoon core temperatures on heat tolerance while subjects are wearing the NBC clothing.

Ingestion of melatonin is also used to induce sleepiness during the daytime (15, 37) and thus in high doses may be counterproductive to extending tolerance time during uncompensable heat stress. A lower repeated dose of 1 mg was used in this investigation, rather than higher 5- or 10-mg doses, to possibly reduce this soporific effect of exogenous melatonin administration.

The purpose of the present study, therefore, was to investigate the effects of two 1-mg doses of melatonin on thermoregulatory responses and tolerance time while subjects were wearing NBC protective clothing during light intermittent exercise in a hot environment. For the placebo trials, it was hypothesized that tolerance time would be reduced for trials conducted in the afternoon. It was also hypothesized that melatonin ingestion would increase tolerance time and that this increase would be greater for trials conducted in the afternoon.

METHODS

Subjects

After receiving approval from the Institute's Human Ethics Committee, nine non-heat-acclimatized men volunteered to participate in the study. Mean values (±SD) for age, weight, height, Dubois body surface area (A_D) and peak O_2 consumption ($\dot{V}O_{2\,peak}$) were 33.6 ± 8.8 yr, 81.5 ± 9.8 kg, 1.76 ± 0.04 m, 1.97 ± 0.13 m^2, and 46.6 ± 6.1 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. The subjects were informed of all details of the experimental procedures and the associated risks and discomforts. After a medical examination to ensure that there were no medical contraindications to their participation in the experiment, each subject gave written informed consent before the first day of data collection.

Determination of Peak Aerobic Power

$\dot{V}O_{2\,peak}$ was determined on a motor-driven treadmill by using open-circuit spirometry (10, 32) before the series of experiments in the climatic chamber started. After 2 min of running at a self-selected pace, the treadmill grade was increased 1%/min, until subjects were running at a 10% grade. Treadmill speed was then increased 0.22 m/s (0.8 km/h) each minute, until the subject could no longer continue. $\dot{V}O_{2\,peak}$ was defined as the highest O_2 uptake ($\dot{V}O_2$) observed during the incremental test. Heart rate (HR) was monitored throughout the incremental test from a telemetry unit (Polar Electro PE3000, Stamford, CT). The HR value, recorded at the end of the exercise test, was defined as the individual's peak value (HR_{peak}).

Experimental Design

All subjects performed four experimental sessions in random order, separated by a minimum of 7 days and a maximum of 14 days. Most trials were performed on a weekly basis for a given subject. The following items were worn during each trial: underwear or jogging shorts, T-shirt, socks, lightweight cotton combat jacket and pants, semipermeable NBC overgarment, jogging shoes, impermeable overboots and gloves, and C4 respirator and cannister. The total thermal resistance of the NBC ensemble determined on a heated copper manikin was $0.29°C \cdot m^2 \cdot W^{-1}$ (1.88 clo), and the Woodcock vapor permeability coefficient (i_m) determined with a completely wetted manikin was 0.33 (19). The experimental sessions involved treadmill walking at 0.97 m/s (3.5 km/h) for 45 min, followed by 15 min of seated rest each hour in an environmental chamber set at 40°C, 30% relative humidity, and a wind speed <0.1 m/s. The experimental trials consisted of two morning and two afternoon sessions, with each trial involving the ingestion of two small capsules containing either placebo (lactose monohydrate) or melatonin (1 mg with lactose monohydrate filler), administered in a double-blind manner. The first capsule was ingested 2 h before entry into the climatic chamber at 0730 for the morning trials and at 1130 for the afternoon sessions. The second capsule was ingested just before beginning of the heat-stress trial at either 0930 or 1330 for the morning and afternoon experiments, respectively. During the 2-h period that intervened between the ingestion of the first and second capsules, subjects were exposed to the same lighting conditions (~500–700 lx), which involved either working at their desk in their office or watching a movie in a laboratory for the first hour and then being instrumented and dressed during the second hour. Subjects were asked to avoid hard exhaustive exercise and the consumption of alcohol or nonsteroidal anti-inflammatory drugs (35) for 24 h and caffeine for 12 h preceding each trial. Each session continued until either rectal temperature (T_{re}) reached 39.3°C, HR remained at or above 95% of HR_{peak} for 3 min, nausea or dizziness precluded further exercise, the subject asked to be removed from the chamber, or the investigator removed the subject from the chamber. Subjects also performed a familiarization trial, which included all aspects of the experimental sessions, with the exception that no capsules were ingested, and used the same criteria for termination of the trial. This session was performed 7 days before the first experimental condition.

Dressing and Weighing Procedures

Subject preparation, insertion of the rectal thermistor, and placement of heat flux transducers have been detailed previously (2). In addition, relative humidity capacitance sensors (Vaisala Sensor Systems, Woburn, MA) and thermistors were taped on the skin and the outer layer of the combat clothing at the upper back, abdomen, and upper thigh. These humidity sensors have an accuracy of ±3%, and the linearity of response was verified for each sensor with saturated salt solutions of lithium chloride, sodium chloride, and potassium sulfate to provide relative humidity measurements of 12, 75, and 97%, respectively. Both nude and dressed weights were recorded before the subjects entered into the chamber. On entering the chamber, the subject's humidity sensors and thermistors, heat flux transducers, and rectal thermistor-monitoring cables were connected to a computerized data-acquisition system (Hewlett-Packard 3497A control unit, 236-9000 computer, and 2934A printer), and the exercise began. Mean values over 1-min periods for T_{re}, a 12-point weighted mean skin temperature ($\bar{T}_{sk}$) and mean heat flow

($\overline{HF}$) (44), and unweighted average skin and garment vapor pressures were recorded and printed by the data-acquisition system. HR was recorded every 5 min from the display on the telemetry receiver. Subjects were given 500 ml of warm water to drink just before entry into the chamber and during the rest periods. The temperature of this water was maintained close to body temperature to reduce the heat-sink effect that cooler water would have on T_{re}. If T_{re} was above 38.7°C, or if the subject did not feel he could continue much longer, water was not provided during the second rest period of the heat-stress trial, since weight measurements after the exposure might not reflect the absorption of this fluid from the intestine. After the completion of each trial, dressed weight was recorded within 1 min after the subject exited from the chamber, and nude weight was recorded after a 5-min undress procedure.

Differences in nude and dressed weights before and after each trial were corrected for fluid intake and respiratory and metabolic weight loss (see below). The rate of sweat production was calculated as the difference between the corrected pretrial and posttrial nude weights, divided by tolerance time, which was defined as the difference in time between removal from and entry into the environmental chamber.

Gas-Exchange Analyses

During each trial, open-circuit spirometry was used to determine expired minute ventilation and $\dot{V}O_2$ by using a 2-min average obtained every 15 min. For all trials, an adaptor was attached to the respirator, which allowed expired air to be collected. Respiratory water loss was calculated by using the $\dot{V}O_2$ measured during the trial and the equation presented by Mitchell et al. (33). Metabolic weight loss was calculated from $\dot{V}O_2$ and the respiratory exchange ratio (RER) by using the equation described by Snellen (39).

Blood Sampling

After the insertion of the rectal thermistor, a 20-gauge catheter (Vialon Insyte, Becton Dickinson) was inserted into an antecubital vein, and a small-bore extension line was connected to the catheter and taped down the forearm to exit at the wrist, which allowed blood sampling to occur when the subject was dressed in the NBC clothing. After the subject had been standing for 15 min following the insertion of the catheter, 10 ml of blood were taken and analyzed for hematocrit (by microcentrifugation) and for plasma melatonin and serum osmolality, calculated from sodium, glucose, and urea nitrogen determinations (Stat Profile Ultra, Nova Biomedical). After this initial blood sample, subjects ingested their first capsule. Subsequent 5-ml blood samples for plasma melatonin analyses were taken 60 min after the ingestion of the first capsule, 115 min after the ingestion of the first capsule and just before the ingestion of the second capsule and entry into the climatic chamber, 40 min into the first treadmill walk, 40 min into the second treadmill walk (or just before exiting the chamber, if the subject could not complete the second treadmill walking period), and after the measurement of the final nude weight.

Determination of Plasma Melatonin

Melatonin was analyzed by using a negative-ion chemical ionization gas chromatograph mass spectrometry-mass spectrometry (GC-MS/MS) technique. D_4-melatonin (Cambridge Isotopes, Cambridge, MA) was utilized as an internal standard. Melatonin was extracted from human blood plasma samples by using solid-phase extractions. The aqueous calibration curve was prepared at concentrations of 10, 50, 100, 200, 500, and 1,000 pg/ml. D_4-melatonin (100 pg/tube) was added to all calibration standards and all samples before their solid-phase extractions. The extractions were done by using methanol-conditioned and water-washed Waters Oasis 1-ml extraction tubes (30 mg hydrophilic lipophilic balanced). One milliliter of standards and plasma samples was applied on the columns, washed three times with 1 ml of distilled water, and subsequently melatonin was eluted two times with 200 µl of methanol. The solvent was removed at room temperature by a stream of nitrogen and briefly put on a freeze-drier to remove all traces of water. The process was done in a darkened room to prevent melatonin degradation. Extracted standards and samples were derivatized by pentafluoropropionic anhydride (100 ml·60°C^{-1}·30 min^{-1}), which was removed at room temperature by a stream of nitrogen, and the dried residues were redissolved in 20 µl of toluene. Two microliters were injected into a Finnigan TSQ 700 GC-MS/MS. The GC-MS/MS conditions were as follows: for GC, a 30-m J&W Scientific DB-5, 0.25-mm ID capillary column, starting at 140°C and ramped to 280°C at 13°C/min; an interface at 280°C; an ion source at 180°C; and the manifold at 80°C. The reagent gas was 5% ammonia in methane at 5,000 mTorr. For negative ion MS/MS, melatonin parent ion m/z 320 and D_4-melatonin m/z 324 were subjected to MS/MS by using argon at an optimal pressure and optimized ionization energy to obtain daughter ions m/z 285, m/z 304, m/z 305 and m/z 289, m/z 308, and m/z 309, respectively. The results were calculated by using a linear-regression calibration curve.

Heat Storage

The rate of heat storage ($\dot{S}$ in W·m^{-2}) was calculated from the heat balance equation

$$\dot{S} = \dot{M} - \dot{W} + (\dot{C} + \dot{R}) + \dot{K} + \dot{C}_{resp} - \dot{E}_{resp} - \dot{E}_{sk} \quad (1)$$

The rate of metabolic heat production ($\dot{M}$) was determined from the measured $\dot{V}O_2$, the RER, and A_D, as

$$\dot{M} = 352(0.23 \cdot RER + 0.77)(\dot{V}O_2 \cdot A_D^{-1}) \quad (2)$$

(see Ref. 36). The external rate of work performed ($\dot{W}$) was considered to be zero, since the subjects either walked on a level treadmill or sat in a chair.

The rate of radiative and convective heat exchange ($\dot{R}$ and $\dot{C}$, respectively) contributed to a positive heat storage, since the chamber temperature exceeded skin temperature. For the walking periods of this study, $\dot{R}$ and $\dot{C}$ were estimated by using the total insulative value of the NBC clothing ensemble (I_T) of 0.291 °C·m^2·W^{-1} (or 1.88 clo), determined at a wind speed of 1.12 m/s on a heated and dry articulating copper manikin (19), and the difference between the chamber temperature of 40°C and $\overline{T}_{sk}$ averaged over each 5-min interval, as

$$\dot{R} + \dot{C} = (40 - \overline{T}_{sk}) \cdot 291^{-1} \quad (3)$$

(see Ref. 20).

For the periods of seated rest, the higher I_T of 0.364 °C·m^2·W^{-1} (2.35 clo) was used for this calculation to reflect the influence of a lack of air movement during rest conditions on the determination of thermal resistance (19).

Conductive heat gain ($\dot{K}$) during the periods of seated rest was estimated from previous work in our laboratory as 3 W·m^{-2} (4).

Respiratory evaporative heat loss ($\dot{E}_{resp}$) and convective heat gain ($\dot{C}_{resp}$) were calculated from the chamber vapor pressure (P_a) of 2.21 kPa for 40°C and 30% relative humidity, and the respired vapor pressure (P_{resp}) of 5.32 kPa, which

assumes 100% saturation of expired air at a mouth temperature (T_{resp}) of 34°C for the chamber conditions (28), as

$$\dot{E}_{resp} = 0.0173 \cdot \dot{M} \cdot (P_{resp} - P_a) \quad (4)$$

and

$$\dot{C}_{resp} = 0.0014 \cdot (T_a - T_{resp}) \quad (5)$$

(see Ref. 16). Evaporative heat loss from the skin ($\dot{E}_{sk}$) was determined from the skin vapor pressure (P_{sk}), the Woodcock vapor permeability coefficient (i_m) of 0.33, I_T, the Lewis relation of 16.5°C/kPa, and P_a as

$$\dot{E}_{sk} = 16.5 \cdot (0.33 \cdot 0.291^{-1}) \cdot (P_{sk} - P_a) \quad (6)$$

(see Ref. 19). P_{sk} was estimated from a model that used the vapor pressure readings obtained with the humidity sensors positioned above the skin surface ($\overline{VP}_{sk}$) and over the combat clothing layer ($\overline{VP}_G$), together with the thermal resistances of the clothing and air layers to generate P_{sk} (8, 32).

Heat storage capacity (S; in kJ/kg) was calculated from $\dot{S}$ and tolerance time as,

$$S = \dot{S} \cdot A_D \cdot \text{mass}^{-1} \cdot (60 \cdot \text{time}) \cdot 1{,}000^{-1} \quad (7)$$

Statistical Analyses

Data are presented as means ± SD. A two-factor (drug and time of day) repeated-measures ANOVA was used to evaluate any differences among the trials for hematocrit, osmolality, sweat production, average metabolic rate, heat storage, and tolerance time. A three-factor (drug, time of day, and time) repeated-measures ANOVA was performed for evaluating the changes in $\dot{V}O_2$, HR, T_{re}, $\overline{T}_{sk}$, $\overline{VP}_{sk}$, and $\overline{VP}_G$ during the exposures. When a significant F-ratio was obtained, a Newman-Keuls post hoc analysis was used to isolate differences among treatment means. For all statistical analyses, the 0.05 level of significance was used.

RESULTS

Indexes of Hydration Status

Nude body weights, hematocrit, and osmolality are reported in Table 1. There were no differences among the trials for any of these dependent measurements, thus indicating that hydration status was similar before the heat-exposures were initiated.

Table 1. *Nude body weight, hematocrit, and serum osmolality before heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing nuclear, biological, and chemical protective ensemble and ingested either melatonin or placebo capsules*

	Morning		Afternoon	
	Melatonin	Placebo	Melatonin	Placebo
Nude body weight, kg	80.0 ± 10.0	80.0 ± 10.0	80.0 ± 9.8	80.1 ± 9.8
Hematocrit, %	44.2 ± 2.9	43.9 ± 3.5	44.0 ± 3.6	43.5 ± 3.7
Osmolality, mosmol/kgH_2O	286.9 ± 1.9	287.0 ± 2.5	287.0 ± 1.3	286.6 ± 2.0

Values are means ± SD; n = 9 men.

Table 2. *Plasma melatonin levels before, during, and immediately after heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing the nuclear, biological, and chemical protective ensemble and ingested either melatonin or placebo capsules*

Time From Start of Heat Stress, min	Morning		Afternoon	
	Melatonin	Placebo	Melatonin	Placebo
−120	18.0 ± 3.9	18.4 ± 9.6	10.0 ± 5.9	11.3 ± 6.6
−60	1,165.3 ± 636.4	15.1 ± 10.2	837.1 ± 343.9	11.6 ± 6.9
−5	857.5 ± 299.7	11.9 ± 9.6	1,129.4 ± 500.6	8.5 ± 5.2
40	1,500.0 ± 618.4	8.5 ± 5.8	1,750.6 ± 550.6	5.0 ± 3.5
100	1,074.9 ± 428.7	11.8 ± 10.8	1,511.0 ± 540.1	7.3 ± 3.5
Post	1,076.9 ± 515.4	9.3 ± 3.7	1,168.4 ± 512.6	7.4 ± 3.6

Values are means ± SD; n = 8 men. Plasma melatonin levels measured in pg/ml.

Plasma Melatonin

Plasma melatonin levels are presented in Table 2. Throughout the morning and afternoon placebo trials, melatonin levels remained below 20 pg/ml. In contrast, plasma melatonin levels increased significantly to ~1,000 pg/ml 60 min after the ingestion of the first 1-mg capsule of melatonin. There was a further significant increase to ~1,600 pg/ml 40 min after the ingestion of the second 1-mg capsule of melatonin. By the end of the heat-stress exposure, plasma melatonin levels had decreased significantly to ~1,100 pg/ml. There was no indication that these elevated melatonin levels were associated with a greater decrease in alertness during the heat stress test, as indicated by responses to the Stanford Sleepiness questionnaire (data not shown).

Indexes of Heat Strain

Metabolic rate. The metabolic rate averaged throughout the heat-stress exposure was not significantly affected by the time of day or ingestion of melatonin (Table 3). Values approximated 144 $W \cdot m^{-2}$.

Rate of sweat production and evaporation. The rate of sweat production was not different during the morning or afternoon trials, nor was sweat rate influenced by the ingestion of melatonin (Table 3). The rate of sweat evaporation, calculated from the humidity sensor data and the model described by Cain and McLellan (8), revealed no effect of melatonin ingestion or the time of day (Table 3).

HR. Figure 1 presents the changes in HR during the morning or afternoon trials. Values increased progressively during the 45-min treadmill walking periods and exceeded 150 beats/min during the second hour of exercise. There were no effects of time of day or melatonin ingestion on the HR response.

T_{re}. During the 2-h period before heat-stress exposure, T_{re} was significantly elevated during the afternoon trials (Fig. 2). The initial T_{re} recorded at the beginning of the heat-stress test was significantly higher during the afternoon (37.1°C) compared with the morning (36.8°C) trials, and this difference between the morning and afternoon tests remained for the duration of the exposure (Fig. 2).

Table 3. *Metabolic rate, rates of sweat production and sweat evaporation, final skin and rectal temperatures, time required for a 1.0°C increase in rectal temperature, tolerance time, rate of heat storage, and the heat storage capacity for the heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while the subjects were wearing nuclear, biological, and chemical protective ensemble and ingested either melatonin or placebo capsules*

	Morning		Afternoon	
	Melatonin	Placebo	Melatonin	Placebo
Metabolic rate, $W \cdot m^{-2}$	145.0 ± 15.8	143.5 ± 19.5	142.9 ± 17.0	144.8 ± 11.6
Sweat production rate, $kg \cdot h^{-1} \cdot m^{-2}$	0.449 ± 0.136	0.424 ± 0.164	0.430 ± 0.151	0.471 ± 0.144
Sweat evaporation rate, $W \cdot m^{-2}$	49.0 ± 12.4	52.8 ± 14.2	50.8 ± 12.3	53.6 ± 13.6
Final skin temperature, °C	37.9 ± 0.3	37.8 ± 0.4	38.1 ± 0.3	38.0 ± 0.3
Final rectal temperature, °C*	39.04 ± 0.27	38.97 ± 0.27	39.22 ± 0.15	39.08 ± 0.25
Time for 1.0°C increase in rectal temperature, min	56.0 ± 12.3	61.2 ± 12.9	61.2 ± 13.8	63.3 ± 10.7
Tolerance time, min	107.6 ± 19.6	111.0 ± 22.0	109.9 ± 23.3	107.2 ± 19.0
Rate of heat storage, $W \cdot m^{-2}$	103.9 ± 23.7	100.6 ± 24.0	100.6 ± 21.8	96.5 ± 21.2
Heat storage capacity, kJ/kg	16.1 ± 2.4	16.1 ± 2.9	16.0 ± 3.5	15.1 ± 3.0

Values are means ± SD; n = 9 men. * Significant difference between the morning and afternoon trials.

Melatonin had no impact on the T_{re} response during either the 2-h period before the heat exposure or when the protective clothing was worn in the chamber at 40°C. The T_{re} recorded at the end of the heat exposure was also significantly higher during the afternoon (39.2°C) compared with the morning (39.0°C) trials (Table 3).

$\overline{T}_{sk}$. The time of day also affected $\overline{T}_{sk}$, with significantly higher values being observed both at the beginning and throughout the afternoon tests (Fig. 3). At the end of the trial, however, $\overline{T}_{sk}$ was not significantly increased during the afternoon tests (Table 3). Melatonin had no effect on the $\overline{T}_{sk}$ response.

$\overline{HF}$. $\overline{HF}$ revealed an initial heat gain during the first 25 min of exposure (Fig. 4). Thereafter, heat loss progressively increased, with heat loss being greater during the exercise periods. Overall, $\overline{HF}$ was greater during the afternoon (21.1 $W \cdot m^{-2}$) compared with the morning (18.8 $W \cdot m^{-2}$) tests. Ingestion of melatonin did not influence heat flow.

Heat storage. Both the rate of heat storage and the heat storage capacity were not affected by the time of day or the ingestion of melatonin (Table 3).

Indexes of Heat Tolerance

Table 4 presents the end-point criteria for termination of the trials. For the 36 trials, exhaustion was

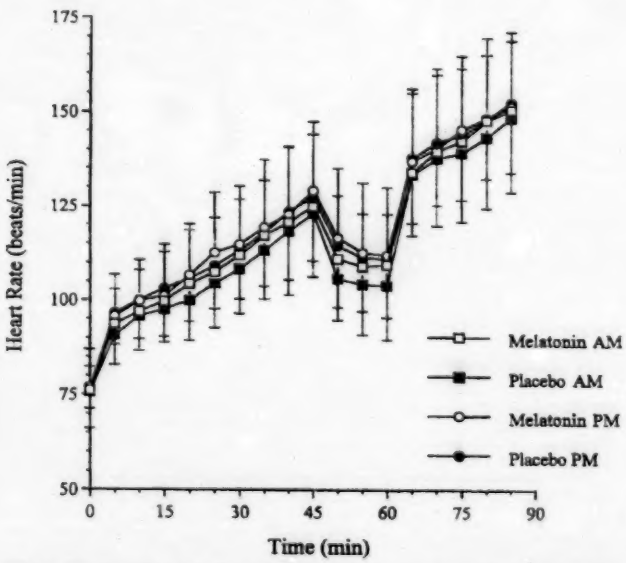

Fig. 1. Changes in heart rate during heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing nuclear, biological, and chemical (NBC) protective ensemble and ingested either melatonin or placebo capsules. Values are means ± SD for n = 9 men. There was no effect of time of day or melatonin ingestion.

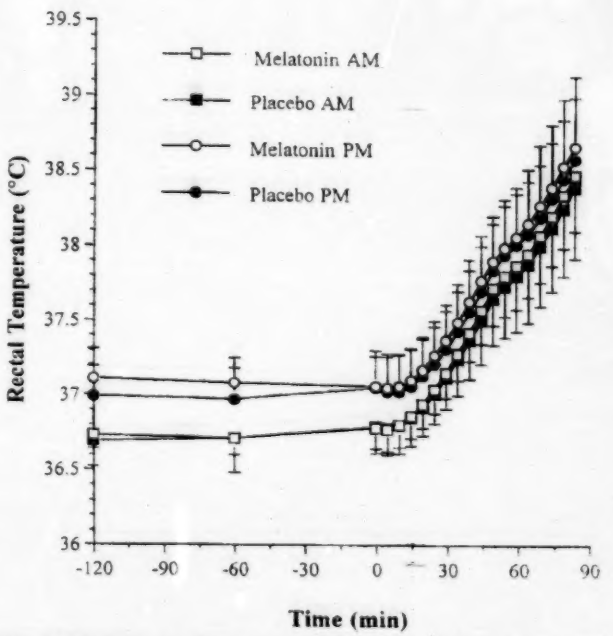

Fig. 2. Changes in rectal temperature during heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity, while subjects were wearing NBC protective ensemble and ingested either melatonin or placebo capsules. Values are means ± SD for n = 9 men. Rectal temperature was significantly increased throughout afternoon trials, and there was no effect of melatonin ingestion.

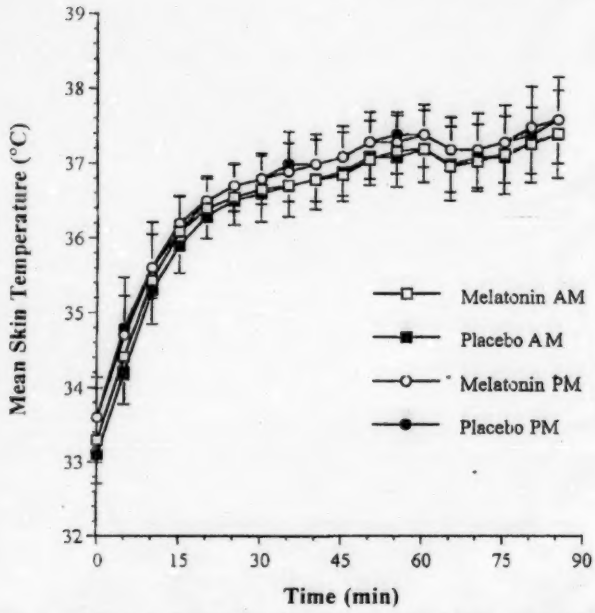

Fig. 3. Changes in mean skin temperature during heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing NBC protective ensemble and ingested either melatonin or placebo capsules. Values are means ± SD for $n = 9$ men. Mean skin temperature was significantly increased for afternoon trials, and there was no effect of melatonin ingestion.

stated as the reason for ending the exposure for 25%, or 9, tests. For seven of these nine trials, T_{re} exceeded 38.8°C, thus indicating that a substantial increase in body heat storage had occurred. For each of the eight tests ended because of subject's dizziness or nausea, T_{re} exceeded 38.8°C. For two of the three tests terminated because of the HR response exceeding 95% of the individual's HR_{peak}, T_{re} exceeded 38.8°C. Tolerance time was not affected by either the time of day or the ingestion of melatonin, with values approaching 110 min for all trials (Table 3).

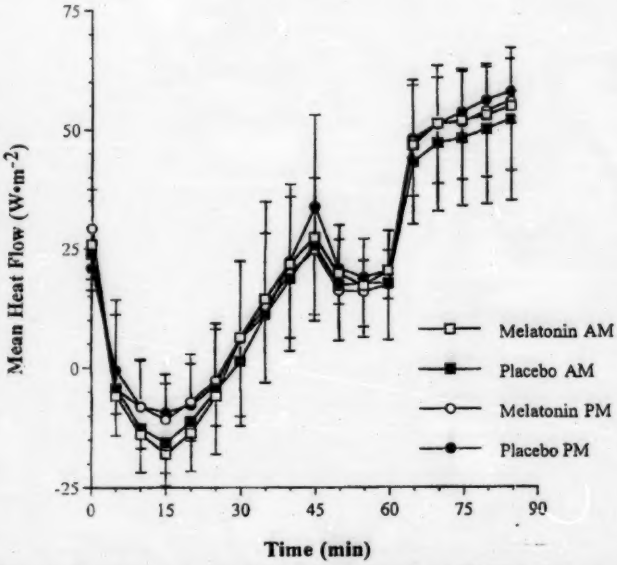

Fig. 4. Changes in mean heat flow during heat-stress trials conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing NBC protective ensemble and ingested either melatonin or placebo capsules. Values are means ± SD for $n = 9$ men. Mean heat flow was significantly increased for the afternoon trials, and there was no effect of melatonin ingestion.

Table 4. *Reasons for termination of heat-stress trial conducted in the morning or afternoon at 40°C and 30% relative humidity while subjects were wearing nuclear, biological, and chemical protective ensemble and ingested either melatonin or placebo capsules*

	Final T_{re}, °C			
	Morning		Afternoon	
Subject No.	Melatonin	Placebo	Melatonin	Placebo
1	T_{re}	T_{re}	T_{re}	T_{re}
2	Exhaustion	Exhaustion	HR	T_{re}
3	Nausea	HR	Dizziness	HR
4	T_{re}	Exhaustion	T_{re}	T_{re}
5	T_{re}	Exhaustion	T_{re}	T_{re}
6	Exhaustion	Exhaustion	Exhaustion	Exhaustion
7	Dizziness	Dizziness	T_{re}	Dizziness
8	T_{re}	Nausea	T_{re}	Nausea
9	T_{re}	Dizziness	T_{re}	Exhaustion

Rectal temperature (T_{re}) represents 39.3°C, and heart rate (HR) represents ≥95% of maximal HR.

DISCUSSION

Two main findings have evolved from the present study. First, the two repeated 1-mg doses of melatonin had no impact on heat tolerance or the thermoregulatory responses during the uncompensable heat stress. Second, trials conducted in the early afternoon were associated with an increased T_{re} tolerated at exhaustion that offset the circadian influence on resting T_{re}, and thus maintained tolerance times similar to trials conducted in the morning.

The 1-mg doses of melatonin were chosen for the present investigation not only because of the reported hypothermic effect (5, 13) but also because we wished to minimize the soporific effects of higher doses (15). The hypothermic effect following the ingestion of 1–10 mg of melatonin has been well documented in humans (5–7, 13–15, 37, 41). Dawson et al. (13) reported a fall in T_{re} of 0.2°C within 2 h after the ingestion of a single 1-mg dose of melatonin, and these authors and others (6) have suggested that core temperature responds to a threshold concentration of melatonin rather than following a dose response. Thus we expected to observe a decrease in T_{re} before beginning the heat-stress trials, which was 2 h after the ingestion of the first 1-mg dose of melatonin. Despite significant increases in plasma melatonin levels (see Table 2), comparable to values reported by others (7, 13) and presumably above a threshold concentration, T_{re} was not different between the melatonin and placebo trials at the beginning of either the morning or afternoon trials.

Most of the studies cited above (5–7, 13, 15, 37, 41), but not all (14), have involved controlled bed rest to eliminate the influence of activity, exposure to bright

light, and changing posture on core temperature. In the present study, subjects' activity levels were only marginally elevated above resting levels during the 2-h period that followed their first 1-mg dose of melatonin. During this time, they watched a movie or worked at their desk during the first hour and then were instrumented and dressed during the second hour. Exposure to bright light during the daytime has no effect on the circadian core temperature response (17), and others have shown the hypothermic effect of low doses of melatonin with simultaneous exposure to a light intensity >1,000 lx (6). Thus we do not feel that exposure to light intensities between 500 and 700 lx in the present investigation masked the hypothermic effect of the melatonin. Subjects remained in a seated position for most of the first hour and then stood for most of the second hour before entering the environmental chamber. Recent findings by Kräuchi et al. (24) have revealed that an orthostatic challenge in changing from the supine to the upright position negated the hypothermic effect of a 5-mg dose of melatonin. Although this orthostatic challenge is far reduced in changing from the sitting to the standing position, Tikuisis and Ducharme (43) have documented a 0.2°C increase in T_{re} for the standing position compared with sitting. During the 2 h before exposure to the heat in the present investigation, T_{re} increased from 36.69 to 36.79°C and from 36.99 to 37.05°C for the morning and afternoon placebo trials, respectively. We cannot discount, therefore, that these small changes represent postural effects on T_{re} that masked the hypothermic influence of the 1-mg dose of melatonin. From a military perspective, it is unlikely, however, that personnel could eliminate postural effects on T_{re} after the ingestion of melatonin for 1–2 h before donning the NBC clothing.

The hypothermic effect of melatonin ingestion also has been reported to be quite variable (13, 37), with individual T_{re} decreases varying from 0.01 to 0.61°C after a 5-mg dose (37). During the 2-h period following the ingestion of the first 1-mg capsule of melatonin in the present study, individual changes in T_{re} varied from an increase of 0.24°C to a decrease of 0.23°C. Changes in plasma melatonin also varied among individuals during this time period from 346 to 2,000 pg/ml, but there was no relationship between the variation in response of this measure and the change in T_{re} during either the morning or afternoon trials. There was also no relationship between body mass and the increase in plasma melatonin, implying that the absolute 1-mg dose of melatonin did not differentially influence plasma concentrations because of differences in body mass. The individual variability in plasma melatonin levels following the 1-mg dose is comparable to the response reported by Dawson et al. (13), and this variability reflects individual differences in absorption and clearance rates (13). We feel that the nonsignificant effect of the 1-mg dose of melatonin on T_{re} before exposure to the uncompensable heat stress reflects the individual difference in sensitivity to the increase in plasma melatonin levels. Whether higher doses of melatonin would be successful in lowering T_{re} before the exposure to the uncompensable heat stress is not known.

The mechanism(s) responsible for the hypothermic effect following melatonin ingestion is unclear. Melatonin receptors have been detected in the preoptic area of the hypothalamus (26), and, thus, melatonin may exert a direct influence on the thermoregulatory center and its control of body temperature. Melatonin also affects vascular tone (45) and increases convective heat loss, as indicated by the increase in temperature of the metatarsals in chickens (38) and feet of humans (24). The intent of the present study was to promote a fall in core temperature with melatonin ingestion before heat exposure. Thus subjects ingested the drug in a cooler environment that, in theory, would allow an increased convective heat loss and fall in core temperature to occur. In certain military settings, personnel may be protected in cooler environments before donning their protective clothing and being exposed to hot environments. In other scenarios, however, exposure to hot environments for prolonged periods may be a requirement regardless of the clothing that is worn. Avenues for convective heat loss would be markedly reduced (or nonexistent) in these hot environments because of the high ambient temperatures. Thus, if melatonin were to have an influence on core temperature in these conditions, sudomotor activity would have to increase to enhance evaporative heat loss and/or the metabolic rate would have to decrease. Both of these responses would imply a direct effect of melatonin on the thermoregulatory center. Certainly, while the subjects were wearing the NBC clothing in the present study, there was no evidence that the metabolic heat production was decreased or sweat rates were increased after the ingestion of 1 mg of melatonin.

Ingestion of melatonin has also been used to induce sleepiness during the daytime (15, 37), and thus we were concerned that this soporific effect of the drug might impact negatively on tolerance time during the uncompensable heat stress. The two 1-mg doses of melatonin were chosen to minimize this potential side effect, and, indeed, there was no indication that subjects were less alert during the heat-stress trials following melatonin ingestion (data not shown). These findings, together with the lack of the hypothermic effect of the drug, have prompted the initiation of additional work in which higher doses of melatonin were used.

Factors such as aerobic fitness (11) and heat acclimation (3, 30), which lower resting core temperature, are associated with increased tolerance time during uncompensable heat stress. Conversely, factors such as mild hypohydration (11, 12) or the postovulatory phase of the menstrual cycle (22, 42), which raise resting core temperature, reduce tolerance time. As a result, we hypothesized that the circadian influence on the initial resting T_{re} would decrease tolerance times during the uncompensable heat stress. The fact that T_{re} tolerated at exhaustion was increased during the afternoon trials was unexpected. Clearly, our hypothesis must be rejected at this time. To our knowledge, no one has examined the circadian influence on the core temperature tolerated at exhaustion during uncompensable heat stress. Nor was the present study designed specifically to address this issue, since ethical constraints

restricted the upper limit that T_{re} could reach and subjects had several cues that could provide inference about tolerance time. The fact that gas exchange was measured every 15 min and that 15 min of rest followed 45 min of walking every hour meant that there were enough time cues for subjects, if so inclined, to try to reproduce their exposure time from trial to trial. However, T_{re} at exhaustion during the morning trials was similar or even higher than levels reported for other heat-stress studies conducted during the morning hours with the NBC clothing (11, 27, 34). Our subjects were highly motivated and did not appear to be ending their trials for any reasons other than discomfort or ethical constraints. In addition, when only those subjects were compared ($n = 4$) who ended all heat exposures below a T_{re} of 39.3°C, the significant difference in final T_{re} between the morning (38.8°C) and afternoon (39.1°C) trials persisted. Given that other effector responses, such as the temperature threshold for the onset of sweating and skin vasodilation, occur at higher temperatures in the afternoon compared with the morning (40), it is possible that the T_{re} tolerated at exhaustion is also regulated at a higher temperature in the afternoon. This interpretation implies that it is not an absolute core temperature that the body can tolerate before exhaustion occurs but rather a given increase in body heat content or delta core temperature (see Table 3). Webb (46) has proposed that the body regulates heat content, rather than core temperature. Our data are consistent with his theory of heat regulation.

Other indexes of temperature regulation, such as $\overline{T}_{sk}$ and $\overline{HF}$, were influenced also by the time of day of exposure to the uncompensable heat stress. Skin temperature (and heat flow) exhibits a circadian rhythm; proximal sites (trunk and leg) are in phase with the oscillations described for core temperature, but temperatures of the extremities (hand, foot) are in reverse phase, increasing in the early morning hours when T_{re} falls (24). The direction and magnitude of the oscillations in $\overline{T}_{sk}$, therefore, will reflect the different weightings of the proximal and distal sites in the calculation of $\overline{T}_{sk}$, with the findings from the present study indicating a small increase in $\overline{T}_{sk}$ and $\overline{HF}$ during the afternoon trial, which was maintained throughout the exposure to the uncompensable heat stress.

In summary, the present study has revealed that two 1-mg doses of melatonin failed to reduce core temperature before heat exposure and, subsequently, had no influence on the thermoregulatory and cardiovascular responses to uncompensable heat stress. The data also have shown that the T_{re} tolerated at exhaustion increased during trials conducted in the early afternoon compared with the morning. Because the initial T_{re} also was increased during the afternoon trials and rates of heat storage were unchanged, tolerance times were similar between the morning and afternoon sessions. Partitional calorimetric estimates of heat storage showed a similar increase in body heat content before exhaustion, regardless of the time of day.

The authors thank D. Kerrigan-Brown, S. Tenaglia, J. Pope, and G. Seabrook for technical assistance; B. Cain for expertise in generating evaporative heat-loss data with the thermal model; and K. Butler for the preparation of the placebo and melatonin capsules at the Clarke Institute pharmacy. The time and effort of the subjects in this investigation are greatly appreciated. Melatonin was considered an investigative new drug in Canada at the time this study was conducted, and approval for its use was granted by the Health Protection Branch of Health Canada. Melatonin was provided by Biosynth International, Inc., Naperville, IL.

Present address of G. M. Brown: Clarke Institute of Psychiatry, 250 College St., Toronto, ON M5T 1R8, Canada.

Address for reprint requests and other correspondence: T. M. McLellan, Defence and Civil Institute of Environmental Medicine, Human Protection and Performance Section, PO Box 2000, Toronto, ON, M3M 3B9, Canada.

Received 17 November 1998, accepted in final form 22 March 1999.

REFERENCES

1. **Amos, D. and R. Hansen.** The physiological strain induced by a new low burden chemical protective ensemble. *Aviat. Space Environ. Med.* 68: 126–131, 1997.
2. **Aoyagi, Y., T. M. McLellan, and R. J. Shephard.** Effects of training and acclimation on heat tolerance in exercising men wearing protective clothing. *Eur. J. Appl. Physiol.* 68: 234–245, 1994.
3. **Aoyagi, Y., T. M. McLellan, and R. J. Shephard.** Effects of 6 versus 12 days of heat acclimation on heat tolerance in lightly exercising men wearing protective clothing. *Eur. J. Appl. Physiol.* 71: 187–196, 1995.
4. **Aoyagi, Y., T. M. McLellan, and R. J. Shephard.** Determination of body heat storage: how to select the weighting of rectal and skin temperatures for clothed subjects. *Int. Arch. Occup. Environ. Health* 68: 325–336, 1996.
5. **Cagnacci, A., J. A. Elliott, and S. S. C. Yen.** Melatonin: a major regulator of the circadian rhythm of core temperature in humans. *J. Clin. Endocrinol. Metab.* 75: 447–452, 1992.
6. **Cagnacci, A., R. Soldani, G. A. Laughlin, and S. S. C. Yen.** Modification of circadian body temperature rhythm during the luteal menstrual phase: role of melatonin. *J. Appl. Physiol.* 80: 25–29, 1996.
7. **Cagnacci, A., R. Soldani, C. Romagnolo, and S. S. C. Yen.** Melatonin-induced decrease of body temperature in women: a threshold event. *Clin. Neuroenocrinol.* 60: 549–552, 1994.
8. **Cain, J. B., and T. M. McLellan.** A model of evaporation from the skin while wearing protective clothing. *Int. J. Biometeorol.* 41: 183–193, 1998.
9. **Carter, B. J., and M. Cammermyer.** Emergence of real casualties during simulated chemical warfare training under high heat conditions. *Mil. Med.* 150: 657–665, 1985.
10. **Cheung, S. S., and T. M. McLellan.** Influence of hydration status and fluid replacement on heat tolerance while wearing NBC protective clothing. *Eur. J. Appl. Physiol.* 77: 139–148, 1998.
11. **Cheung, S. S., and T. M. McLellan.** Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. *J. Appl. Physiol.* 84: 1731–1739, 1998.
12. **Cheung, S. S., and T. M. McLellan.** Influence of hydration status and short-term aerobic training on tolerance during uncompensable heat stress. *Eur. J. Appl. Physiol.* 78: 50–58, 1998.
13. **Dawson, D., S. Gibbon, and P. Singh.** The hypothermic effect of melatonin on core body temperature: is more better? *J. Pineal Res.* 20: 192–197, 1996.
14. **Deacon, S., and J. Arendt.** Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. *Brain Res.* 688: 77–85, 1995.
15. **Dollins, A. B., I. V. Zhdanova, R. J. Wurtman, H. J. Lynch, and M. H. Deng.** Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc. Natl. Acad. Sci. USA* 91: 1824–1828, 1994.
16. **Fanger, P. O.** *Thermal Comfort: Analysis and Applications in Environmental Engineering.* Copenhagen, Denmark: Danish Technical Press, 1970.

17. **French, J., P. Hannon, and G. C. Brainard.** Effects of bright illumination on body temperature and human performance. *Ann. Rev. Chronopharmacol.* 7: 37–40, 1990.
18. **Goldman, R. F.** Tolerance time for work in the heat when wearing CBR protective clothing. *Mil. Med.* 128: 776–786, 1963.
19. **Gonzalez, R. R., C. A. Levell, L. A. Stroschein, J. A. Gonzalez, and K. B. Pandolf.** *Copper Manikin and Heat Strain Model Evaluations of Chemical Protective Ensembles for the Technical Cooperation Program (TTCP).* Natick, MA: US Army Research Institute of Environmental Medicine, (Tech. Rpt. No. 94-4, 1993.)
20. **Gonzalez, R. R., T. M. McLellan, W. R. Withey, S. K. Chang, and K. B. Pandolf.** Heat strain models applicable for protective clothing systems: comparison of core temperature response. *J. Appl. Physiol.* 83: 1017–1032, 1997.
21. **Henane, R. J., J. Bittel, R. Viret, and S. Morino.** Thermal strain resulting from protective clothing of an armored vehicle crew in warm conditions. *Aviat. Space Environ. Med.* 50: 599–603, 1979.
22. **Kolka, M. A., and L. A. Stephenson.** Interaction of menstrual cycle phase, clothing resistance and exercise on thermoregulation in women. *J. Therm. Biol.* 22: 137–141, 1997.
23. **Kraning, K. K., II, and R. R. Gonzalez.** Physiological consequences of intermittent exercise during compensable and uncompensable heat stress. *J. Appl. Physiol.* 71: 2138–2145, 1991.
24. **Kräuchi, K., C. Cajochen, M. Hofmann, and A. Wirz-Justice.** Melatonin and orthostasis: interactions of posture with subjective sleepiness, heart rate, and skin and core temperature (Abstract). *Sleep Res.* 26: 79, 1997.
25. **Kräuchi, K., and A. Wirz-Justice.** Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36) R819–R829, 1994.
26. **Krause, D. N., and M. L. Dubocovich.** Regulatory sites in the melatonin system of mammals. *Trends Neurosci.* 13: 464–470, 1990.
27. **Latzka, W. A., M. N. Sawka, S. J. Montain, G. S. Skrinar, R. A. Fielding, R. P. Matott, and K. B. Pandolf.** Hyperhydration: tolerance and cardiovascular effects during uncompensable exercise-heat stress. *J. Appl. Physiol.* 84: 1858–1864, 1998.
28. **Livingstone, S. D., R. W. Nolan, J. B. Cain, and A. A. Keefe.** Effect of working in hot environments on respiratory air temperatures. *Eur. J. Appl. Physiol.* 69: 98–101, 1994.
29. **McLellan, T. M.** Work performance at 40°C with Canadian Forces biological and chemical protective clothing. *Aviat. Space Environ. Med.* 64: 1094–1100, 1993.
30. **McLellan, T. M., and Y. Aoyagi.** Heat stain in protective clothing following hot-wet or hot-dry heat acclimation. *Can. J. Appl. Physiol.* 21: 90–108, 1996.
31. **McLellan, T. M., I. Jacobs, and J. B. Bain.** Influence of temperature and metabolic rate on work performance with Canadian Forces NBC clothing. *Aviat. Space Environ. Med.* 64: 587–594, 1993.
32. **McLellan, T. M., J. I. Pope, J. B. Cain, and S. S. Cheung.** Effects of metabolic rate and ambient vapour pressure on heat strain in protective clothing. *Eur. J. Appl. Physiol.* 74: 518–527, 1996.
33. **Mitchell, J. W., E. R. Nadel, and J. A. J. Stolwijk.** Respiratory weight losses during exercise. *J. Appl. Physiol.* 32: 474–476, 1972.
34. **Montain, S. J., M. N. Sawka, B. S. Cadarette, M. D. Quigley, and J. M. McKay.** Physiological tolerance to uncompensable heat stress: effects of exercise intensity, protective clothing, and climate. *J. Appl. Physiol.* 77: 216–222, 1994.
35. **Murphy, P. J., B. L. Myers, and P. Badia.** Nonsteroidal anti-inflammatory drugs alter body temperature and suppress melatonin in humans. *Physiol. Behav.* 59: 133–139, 1996.
36. **Nishi, Y.** Measurement of thermal balance of man. In: *Bioengineering, Thermal Physiology and Comfort,* edited by K. Cena and J. A. Clark. New York: Elsevier Scientific, 1981, p. 29–39.
37. **Reid, K., C. Van Den Heuvel, and D. Dawson.** Day-time melatonin administration: effects on core temperature and sleep onset latency. *J. Sleep Res.* 5: 150–154, 1996.
38. **Rozenboim, I., L. Miara, and D. Wolfenson.** The thermoregulatory mechanism of melatonin-induced hypothermia in chicken. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R232–R236, 1998.
39. **Snellen, J. W.** Mean body temperature and the control of thermal sweating. *Acta Physiol. Pharmacol. Nederl.* 14: 99–174, 1966.
40. **Stephenson, L. A., C. B. Wenger, B. H. O'Donovan, and E. R. Nadel.** Circadian rhythm in sweating and cutaneous blood flow. *Am. J. Physiol.* 246 (*Regulatory Integrative Comp. Physiol.* 15): R321–R324, 1984.
41. **Strassman, R. J., C. R. Qualls, E. J. Lisansky, and G. T. Peake.** Elevated rectal temperature produced by all-night bright light is reversed by melatoin infusion in men. *J. Appl. Physiol.* 71: 2178–2182, 1991.
42. **Tenaglia, S. A., T. M. McLellan, and P. P. Klentrou.** Influence of menstrual cycle and oral contraceptive use during compensable and uncompensable heat stress. *Eur. J. Appl. Physiol.* In press.
43. **Tikuisis, P., and M. B. Ducharme.** The effect of postural changes on body temperatures and heat balance. *Eur. J. Appl. Physiol.* 72: 451–459, 1996.
44. **Vallerand, A. L., R. Limmer, and I. F. Schmegner.** Computer acquisition and analysis of skin temperature and heat flow data from heat flux transducers. *Comp. Methods Prog. Biomed.* 30: 279–282, 1989.
45. **Viswanathan, M., J. T. Laitinen, and J. M. Saavedra.** Expression of melatonin receptors in arteries involved in thermoregulation. *Proc. Natl. Acad. Sci. USA* 87: 6200–6203, 1990.
46. **Webb, P.** The physiology of heat regulation. *Am. J. Physiol.* 268 (*Regulatory Integrative Comp. Physiol.* 37): R838–R850, 1995.